Chapter:

Paper Chromatography
&
Thin Layer Chromatography
&
Size Exclusion Chromatography

Prepared By:

M JUNAID SAHOO

Lecturer Chemistry in HED (Govt. College)

Chemistry with MJS

Chemistry Preparation by MJS

Chapter #

CHROMATOGRAPHIC TECHNIQUES

* Chromatography is the best separating technique. Deals with the separation of mixture of substances/solutes through the distribution b/w two immisible phaseses on the basis of interaction.
  - stationary phase
  - Mobile phase

* originally it was applied to coloured substances such as plant pigments & Dyes.

  ⇒ Greek word : chroma → colour
  : Graphe → writing

* Later on, it was being applied to colourless substances.

Birth of chromatography:

* Russian Botanist, Michael Tswett, used the column for the separation of plant pigments and introduce the word "chromatography".

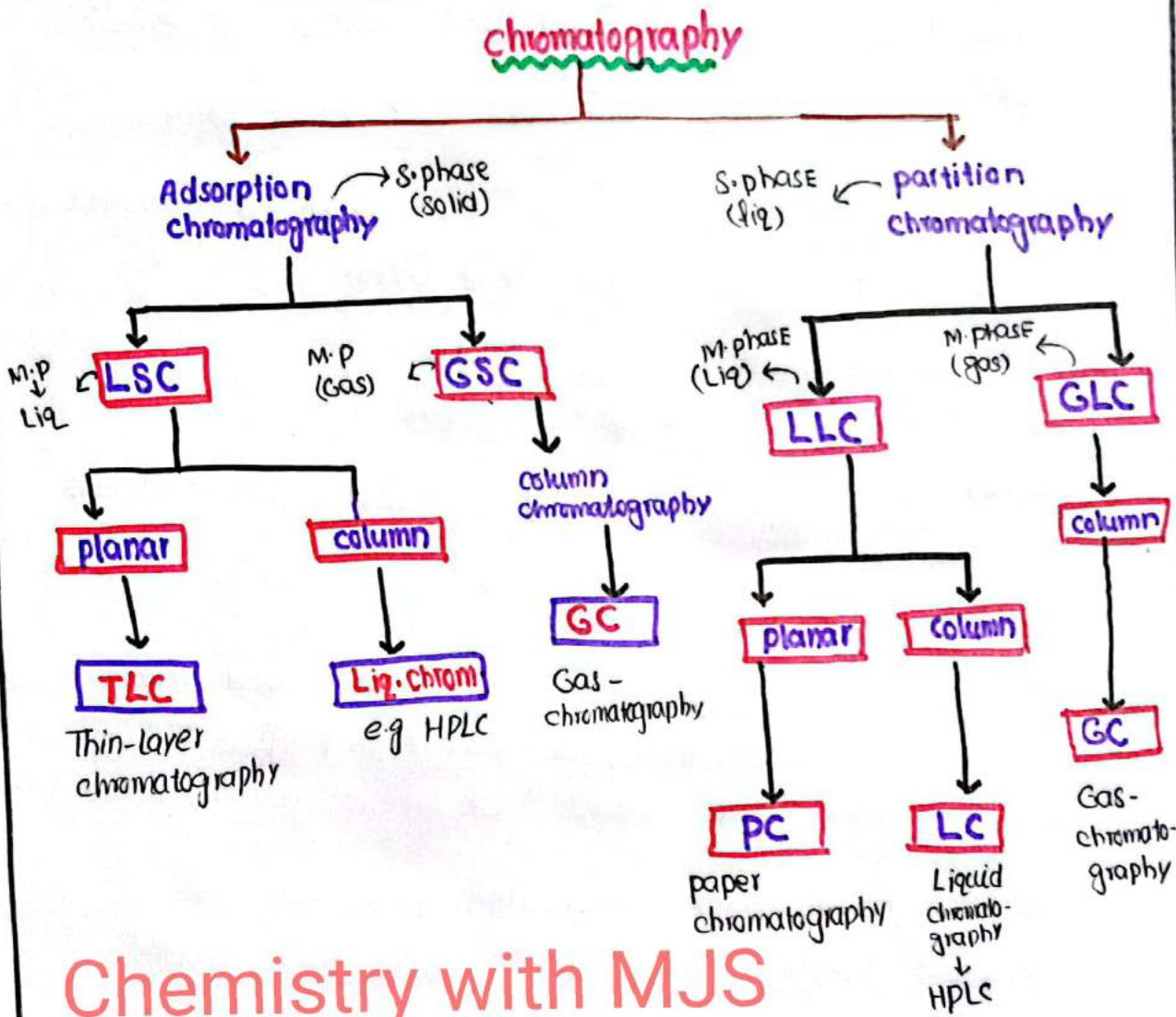
* Strain → define chromatography on the basis of separation of substances by differential migration, Migrating Force was named solvent.

* Chromatography is an extension of Liq-Liq extraction

* More efficient separating technique than solvent Extraction.

- Diversity is more in chromatography than SPE & Liq-Liq Extraction.
- physical Force of interaction observed.
- Dynamic process (Flow process)

V.V.Imp CLASSIFICATION

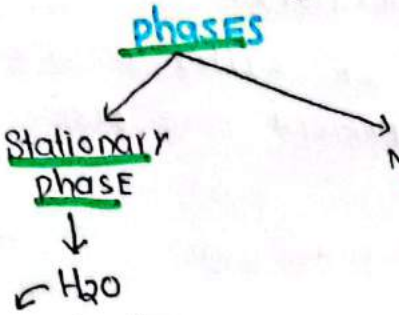


- planar chromatography → paper chromatography & TLC
- column chromatography → HPLC, GC, ion, Exchange
  - ↳ used for Air sensitive compounds.
- Elute → desired substance, want to be separated
- Eluent → substance which separates the solute e.g M.phase
- Elution → process of separation of Analyte/solute

Paper chromatography → partion Chromatography

* Simplest and most widely used separating technique.
* Used to separate mixtures.

classical chromatographic technique



✓ Principle / Theory:

* Separation occurs due to the differential migration of two phases → Diff. in partition coefficients
* Capillary Action is performed → which rises the mobile phase.
* Generally paper chromatography works on partition principle But it can be modified to Adsorption, if paper is impregnated with an adsorbent such as Silica & Alumina.

Rf value: Retardation Factor / Retention factor (R_f)

$$R_f = \frac{\text{Distance travelled by solute/Analyte/component}}{\text{Distance travelled by solvent front}}$$

* Each component in mixture has different R_f value.

Operations involved in paper chromatography:

1) Paper preparation:

Size/thickness
Porosity
Strength of F. paper

~ 0.5 — 1.5 cm width
~ 12 cm Length
} Strip of filter paper

* Size of paper depends on available apparatus in Laboratory.
* Different nature of Filter paper are also available → Different pore size e.g 42, 43, 44
* Mostly What-man Filter paper is used.

2) Preparation of solution / Choice of solvent

* pure liquid is directly applied.
* Solids → make solution in suitable solvent
* Choice of solvent/M-phase is very important.

→ pure single solvent can be used
→ If separations are not Good using single solvent then, use mixture of solvents.

3) Application of Sample:

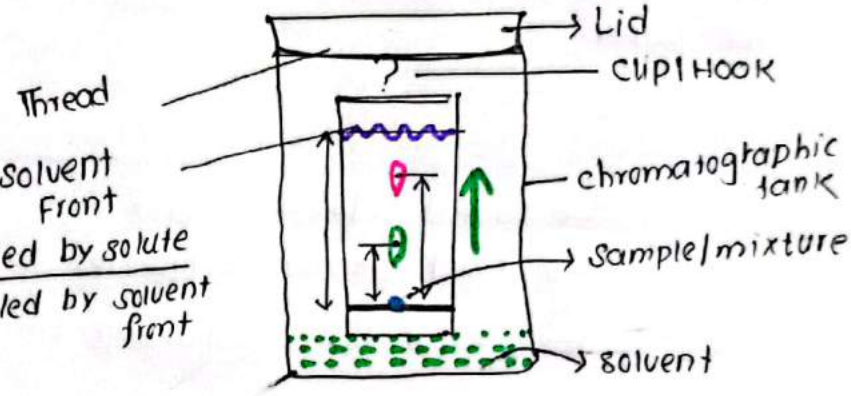
* spot size ~ 5cm
* For Application → use capillary tube
* use diff capillary tubes for diff. samples.
* spot should be conc. But small size (min. volume) be
* If large volume used → spot will diffused & poor separation

4) Developement of chromatogram:

There are different ways to develop the chromatogram.

(i) Ascending
(ii) Descending
(iii) Radial/circular
(iv) One-Dimentional.
(v) 2-Dimentional.

∴ **Ascending paper chromatography:** → solvent moves upward
↳ (Capillary Action is performed)



(chromatogram)

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

* paper strip should not touch to the wall of chromatographic tank
* Hang the paper strip straight
* If solvent front is not straight then takes it mean or middle point.
* paper strip should not dipped in solvent only touch to the solvent surface
* Measure the diftance from base line to the mid point of sample/component.

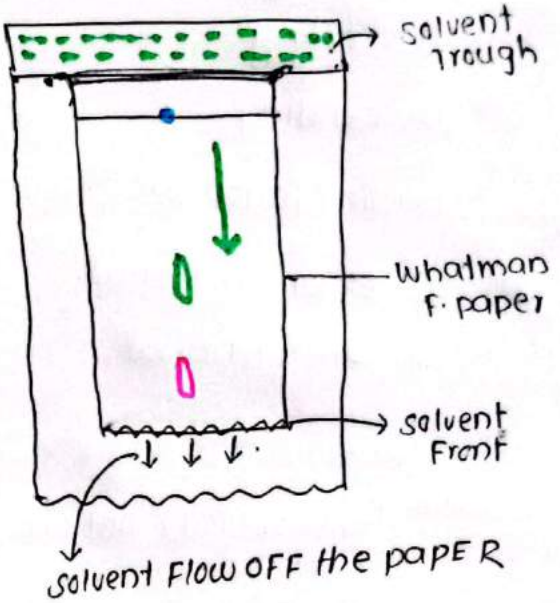
limitation: (i) Not suitable for slow moving solvent
(ii) Not suitable for those having low R_f value

: Descending paper chromatography → solvent moves downward

↳ (Capillary Action + Gravitational Force)

★ Fast and Rapid → technique than Ascending)

* Fast & Rapid
* USED For those solvent whose Rf values are Lower.
* Separation is not Good BIC solvent does not take time to sample to interact with s.phase.
* Solvent moves Fastly downward & flow off the paper.



: Radial / circular paper chromatography:

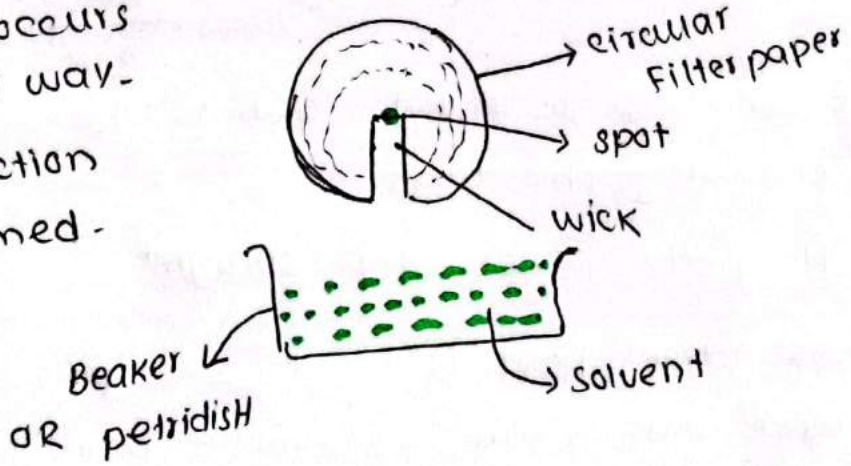
↳ Radial development

↳ Fast technique / Better Results

↳ USEd for Substances having Low Rf value.

⇒ Separtion occurs in circular way.

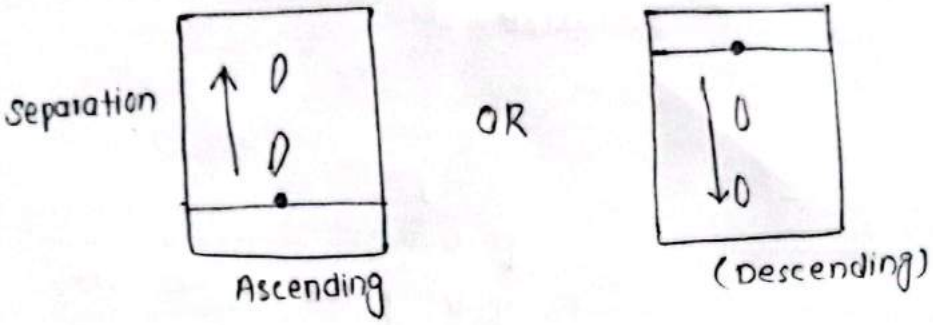
⇒ capillary Action is performed.



One-Dimentional-chromatography

→ Also called Horizontal chromatography

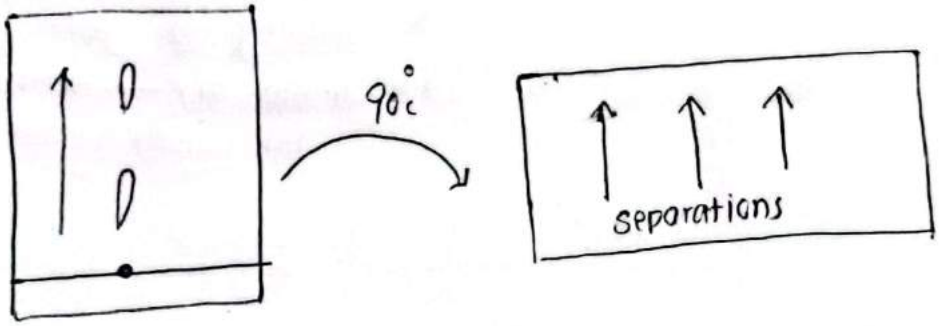
→ Separations occcurs in only one direction (Not good separation)



Two-Dimentional-chromatography

↳ Best performance than 1-Dimentional

↳ used for separating complex mixtures of compounds having same polarity



- 1st separation occurs in one direction & then rotate the paper at 90° and Allow the separation in another direction thus Better separations are performed.
- Highly preffered on 1-Dimentional
- Solvent pass through the Filter paper more than one-time

5) Detection of spots:

* If spots are coloured → then no need to visualize it by any method
* If spots are colourless → then it is important to visualize it.

physical methods

* Fluorescence
* Radioactivity
* UV- Lamp
* Drying the paper
  ↓sometime colour visualized.

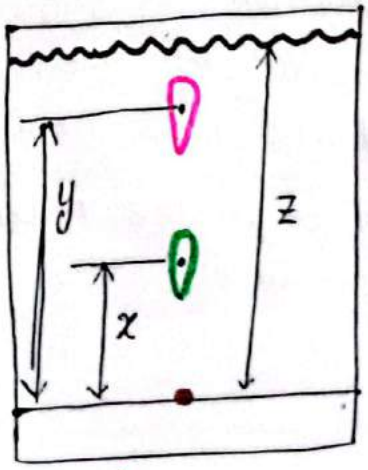
chemical methods

* spraying the Agents on chromatogram
  e.g For Metal → Detection we use DMG which make the colorful product.
* pass → NH_3 gas throughout paper
* NH_4OH can also be used.
* passing H_2 gas.
* Simple DRY → Nature change thus colour observed.

6) Calculation of R_f value: / PERFormance

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by solvent Front}}$$

make the centeral point



∴ For A (Green)

$$R_f = \frac{x}{z}$$

∴ For B (Red)

$$R_f = \frac{y}{z}$$

: Rf value Gives the valuable information:

IF X ∴ $R_f = 1$ → then solute has no affinity for stationary phase and moves with M phase.
So → NO Better separation → General not possible

✓ ∴ $R_f < 1$ → Rf value always less than 1- BIC solvent alway cover more distance than solute (Better for separation)

X ∴ $R_f > 1$ → Not possible BIC solute can not move Faster than solvent.

X ∴ $R_f = 0$ → solute completely Remains with stationary phase thus become immobile
NIO → separation occurs.

Range: 0 —— 1 (R_f)

∴ $R_f > 0$ OR $R_f < 1$

Factors Affecting on Rf value:

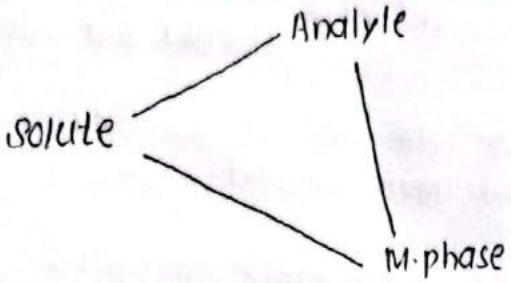
* **Amount of solute:**
  - High volume ↑ more dispersed ↑ Thus not Good separation
  - USE Low volume → small drop by capillary tube.

* **Nature of solute:** ⇄ polar / non-polar

in paper chromatography, stationary phase is polar, it is called Normal phase chromatography

* If our solute is polar then it will be more interacted to stationary phase, thus separation will not occur.
For this, we have to take more polar solvents to break this interaction.
→ Thus solute will travelled less distance
Rf value will decrease (lower)

★ If our solute is non-polar, → Having some polar character interact with water for Functionalization, thus we will use Non-polar solvents, which move away the Solute & separate it.



For Efficient Separation;

★ Nature of Solute should more resemble to solvent

★ Stationary phase & M.phase polarity should different.

⇒ If we Reverse the polarity of Stationary phase in paper chromatography (H_2O) then Stationary phase will become Non-polar ⟶ called Reverse phase chromatography.

↓ How to convert?

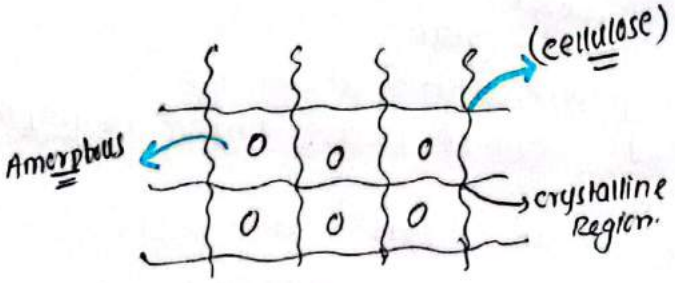
⇒ Dry the paper so 1st water of Amorphous Region evaporate

⇒ Further dry thus, crystalline H-bonding Region evaporate

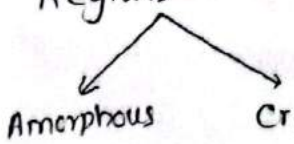
Thus paper will act as non-polar Regions

↓

Stationary phase



paper has two Regions



★ Nature of M. phase (solvent)

- Nature polar/non-polar
- Ratio of solvents
- solvent interaction with solute
- solvent interaction with S phase

If two are more than two solvents then Ratio is very important. e.g

Benzene : H_2O : Acetone

✓ For Better separation Ratio varies thus Rf value change

★ Temperature:

If T ↑↑ then Mobile phase Rapidly moves, so it will give no time to Solute interact with stationary phase, thus Mixture will not separate.

★ poor Resolution/separation observed by T ↑

★ Pressure:

★ pressure indirectly Affects the performance in paper-chromatograph BIC this is performed under Atmospheric pressure.

★ If Solvent Flow is too fast due to increased pressure, it may lead to poor separation & inaccurate Rf values.

★ Structure of paper:

should
- clear
- clean.
- porosity
- Avoid from dirt & dust
- Mineral contents in paper
  - More mineral contents causes more ASH, make unsuitable for paper chromatography

Applications

(i) Chemical Analysis:

- Used For Qualitative & Quantitative analysis OF Mixtures.
- To identify the components present in a Sample.

(ii) Forensics:

- To Analyze ink composition
- To identify Dyes, drugs, poisons in Biological Samples.

(iii) Biochemistry:

- To study Amino acids, separation OF Amino-acids
- Ninhydrin used to locate the A. Acids.

(iv) Separation of cations & Anions:

- Used to separate different cations & Anions From mixtures.

Thin Layer Chromatography

↳ Adsorption chromatography

Superiority

* TLC is more superior separating technique than the paper chromatography.

BIC

- More efficient separation due to Adsorption
- Highly sensitive technique

Stationary phase: → solid (Adsorbant material)

e.g Silica, Alumina, cellulosic powder Silicates, polymers, Gel etc.

Mobile phase: → Liquid

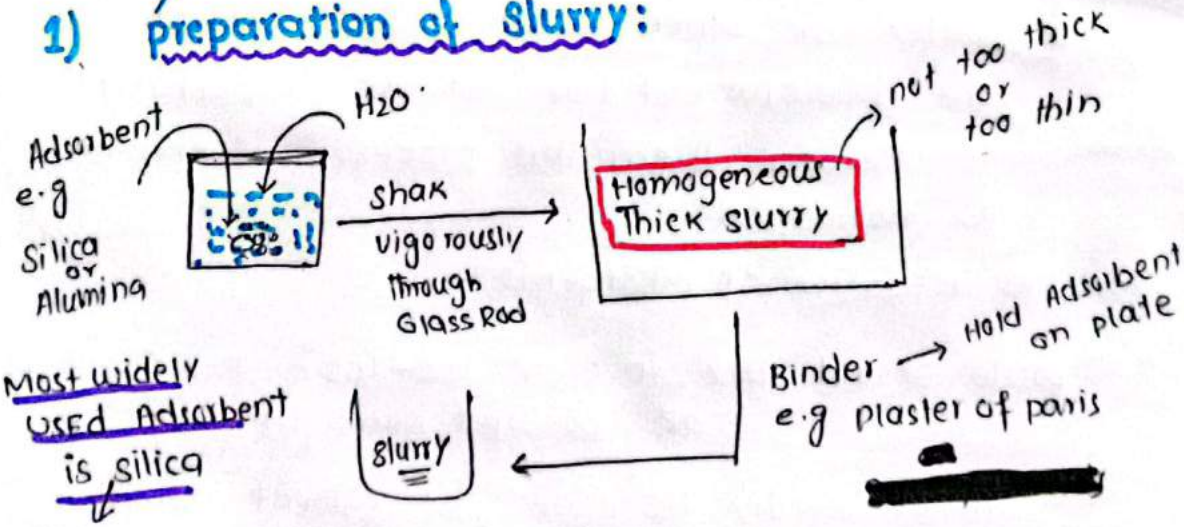
principle / Theory:

The basic principle based on Adsorption phenomena (physical interactions)- Mobile phase separate the solute, when percolates through the Adsorben on the basis of interaction.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

operations involved in TLC

1) preparation of Slurry:



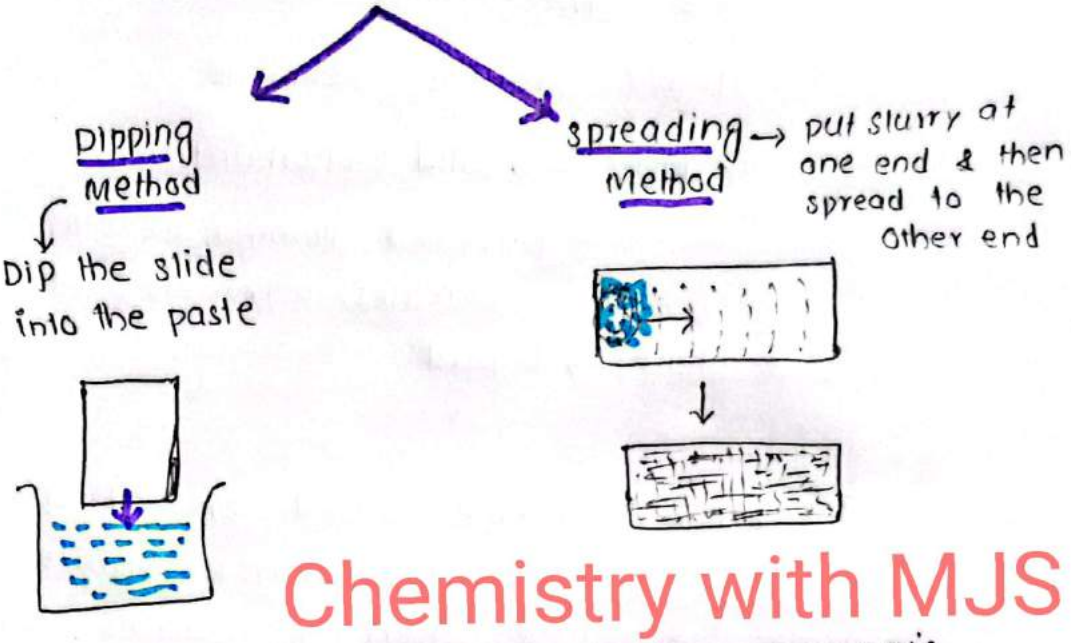
For ★ Bases & steroids → Alumina

For polar compound e.g Aminoacids & Nucleotides → cellulose powder

2) Preparation of plate for developement:

- plate may be plastic or Glass type
  - → Glass sheet → most widely used
  - → plastic sheet.
  - → metal sheet
  - → wood-slate

To make thin layer on plate two methods are used



★ Generally we use two clean microscopic slides together to make a double thickness of glass.

★ Two slides dipped together into the slurry

3) Drying of plates/Activation:

★ water or other polar solvents Affect the development, so it is important to Remove them. This process is known as Activation.

For the activation of Adsorbents;

★ plate should Dry in oven 100-105°c For 30 minutes OR powerful sun light.

★ Do not overdry the plate just solvent should evaporate.

4) Application of Sample:

- line draw on the back side of plate & insert the samples through capillary tube or micropipette
- Sample should not diffused.

5) Developement of chromatogram:

↳ Mostly Ascending technique is used
↳ Developement is same just like paper chromatogram

6) Detection of spots:

colourless substances are detected by using Locating Agents → which produce colours.

* Some Aromatic & Non-Aromatic compound shows dark spot → thus inspect under UV-light
* I_2 → locating Agent → imparts dark brown colour.
  OR
  10% methanolic solution of Iodine.
* H_2SO_4 Also a good locating agent
* H_2SO_4 + Oxidizing agents → Better Results.
  ↓
  e.g $KMnO_4$, HNO_3, chromic acid etc.
* For the detection of spots → Locating agents are sprayed

7) Calculation of R_f value:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Factors are discussed already in paper chromatography

✔ Applications of TLC:

TLC technique is very sensitive & gives sharp & better Resolution

* purity:

purity of sample can be carried out by TLC in organic synthesis.

- Single spot on developing plate → Single product
- More than one spot → Formation of By-product
- product can be confirmed by comparing R_f values of the product with that of Authentic sample.

* identification:

Plant extracts, drugs, adulterants can be identified in food products.

* impurities:

in pharmaceuticals, TLC useful to detect the impurities.

* Separation of Metals:

TLC used to separate the different metals e.g Ni, Co, Mn & Zn etc.

* Reaction Rate:

Reaction progress can Also be monitored by TLC. Either Reaction is completed or not.

SIZE EXCLUSION CHROMATOGRAPHY

SEC | GPC → Gel permeation chromatography

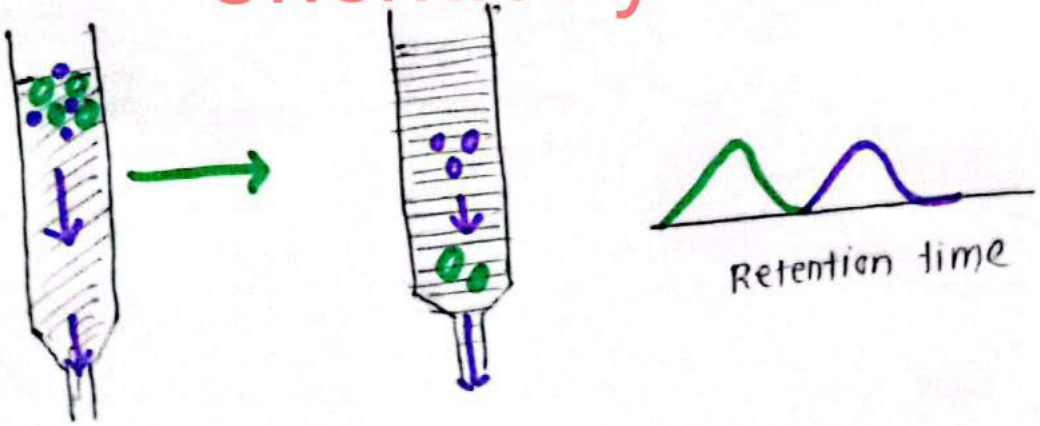
→ ★ Separation occurs on the basis of size of solute particles.

★ Highly porous stationary phase is used.
e.g polymer matrix (Hydrated polymer Beads) → porous

★ Also a Liquid chromatography.
↳ Liquid mobile phase is used.

★ Larger molecules eluted 1st bc they can not enter into the pores / not entangled into pores so less Retention time is Required → easily separated

★ Smaller molecules eluted last bc they pass through the pores, interact with the stationary phase & entangled, thus more time is Required to mobile phase to carry / exil the molecules from pores So more Retention time → separated late.



Applications:

* **protein purification:**

SEC separates proteins on their size, allowing for the separation of specific target proteins from mixture.

* **polymer characterization:**

SEC is used to determine the molecular weight distribution of polymers which is crucial in various industries such as plastics and materials.

* **Environmental analysis:**

to study the size distribution of organic & inorganic particles in environmental samples e.g water sample.

* **Quality control:**

Molecular size distribution of compxnents in food & baverages.

* **characterization of Nanoparticles:**

on the basis of size Nano-particles are separated → Nanotechnology Research

BEST OF LUCK

MJS